

# Selection for Imidacloprid Resistance in Silverleaf Whiteflies from the Imperial Valley and Development of a Hydroponic Bioassay for Resistance Monitoring

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(Received 15 July 1996; revised version received 11 April 1997; accepted 4 August 1997)

**Abstract:** A field-collected population of the silverleaf whitefly, *Bemisia argentifolii*, was selected with the nicotinyl compound, imidacloprid, over 32 generations to determine if resistance would develop when maintained under continuous selection pressure in a greenhouse. Resistance was slow to increase at first with low to moderate levels of resistance (RR from 6- to 17-fold) in the first 15 generations of selection. Further selection steadily led to higher levels of resistance, with the greatest resistance ratio at 82-fold, the gradual rise suggesting the involvement of a polygenic system. At the end of the selection, slopes of probit regressions were substantially steeper than earlier, indicating increased homogeneity of imidacloprid resistance in this strain.

A hydroponic bioassay featuring systemic uptake of imidacloprid through roots was developed to monitor the changes in resistance to imidacloprid in the selected whitefly strain and in seven field-collected strains from Imperial Valley, California. Six out of seven field-collected strains exhibited low  $LC_{50}$  values (0.002 to 0.512 mg ml<sup>-1</sup>) compared to the selected resistant strain, with one exception where the  $LC_{50}$  was 0.926 mg ml<sup>-1</sup> (RR = 15.0). Variation in responses to imidacloprid in the field strains suggest that this technique is sufficiently sensitive to detect differences in susceptibilities of whitefly populations. The imidacloprid-resistant strain showed no cross-resistance to endosulfan, chlorpyrifos or methomyl (RR ranging from 0.4- to 1.5-fold). A low level of cross-resistance was observed to bifenthrin in the IM-R strain at 7-fold. The success of selection for resistance to imidacloprid has serious implications for whitefly control programs that rely heavily on imidacloprid.

Pestic. Sci., 51, 419–428, 1997

No. of Figures: 1. No. of Tables: 4. No. of Refs: 27

Key words: *Bemisia argentifolii*, whiteflies, imidacloprid, selection of resistance, resistance monitoring, cross-resistance

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Contract grant sponsor: Bayer AG.

## 1 INTRODUCTION

The world-wide emergence of the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring,<sup>1</sup> as a major pest of agricultural and floricultural crops represents a serious challenge to the broader pest-management community. For growers and pest-control advisors, control measures directed against the biotically explosive silverleaf whitefly can be strained to the limit, especially in situations where this broadly polyphagous pest multiplies on a succession of crops.<sup>2</sup> Expansion of whitefly populations beyond the regulatory capacity of natural enemies leaves little choice for growers but to protect their crops with insecticides. As the principal pest species in many production systems worldwide, *Bemisia* spp. whiteflies have demonstrated high resistance levels<sup>3–5</sup> to insecticides as well as an extraordinary capacity to expand to new crops and attain even higher levels of pest status. For example, after the emergence of *Bemisia tabaci* (Genn.) as a primary cotton pest in the early 1980s in California's Imperial Valley, far more serious pest status resulted when *Bemisia argentifolii* displaced *B. tabaci* in the early 1990s to become a multi-crop pest by attacking additional field and vegetable crops.<sup>6</sup>

One of the most encouraging developments in whitefly management was the introduction of imidacloprid. Representing a novel class of insecticide, the chloronicotinyls, imidacloprid is a highly effective systemic and contact insecticide against sucking insects such as aphids, leafhoppers, planthoppers and whiteflies.<sup>7</sup> In the Imperial Valley, a single at-planting application of imidacloprid offers protection to lettuce and cole crops for 45–60 days even under extreme whitefly pressure during late summer–early fall.<sup>8</sup>

The availability of imidacloprid products for many crops has advantages beyond effective whitefly control. As new chemistry, imidacloprid provides an important alternative to the pyrethroids, organophosphates and cyclodienes that have been heavily used for whitefly management. The season-long control with imidacloprid in fall vegetables and spring melons results in fewer treatments than with traditional insecticide sprays. Moreover, as a systemic insecticide, imidacloprid probably has a much lower negative impact on the natural enemy complex of whiteflies<sup>7</sup> than contact insecticides.

Systemic insecticides, including imidacloprid, generate higher selection for resistance under prolonged residual persistence and higher exposure levels<sup>9</sup> than do foliar sprays. The soil application of imidacloprid gives longer persistence in the crop than do foliar sprays<sup>10</sup> and all stages of the pest receive a prolonged exposure to imidacloprid. When coupled with the long-term persistence of imidacloprid in plants, the selection pressure for resistance development is likely to be higher than for contact insecticides.

Selection of whitefly strains resistant to imidacloprid in the laboratory may provide an indication of the genetic potential for resistance development in natural populations, and may be useful for formulating a comprehensive resistance-management program. This study was undertaken mainly to investigate the resistance potential of a field population of *B. argentifolii* under rigorous selection pressure with imidacloprid over successive generations in a greenhouse.

Upon developing a resistant strain, our interests were expanded to include three objectives. The first objective was to establish a baseline response of whitefly populations to imidacloprid at an early stage in its commercial usage. The ability to detect changes in responses of target populations is essential for tracking the performance of an insecticide over time. To accomplish this, the second objective was to develop a dependable bioassay technique that reflected the mechanism of systemic uptake of imidacloprid confronting whiteflies in their crop environment, and one sensitive enough to detect slight changes in response to different concentrations of imidacloprid. The third objective was to investigate whether selection with imidacloprid may confer resistance to other chemicals from different classes of chemistry, such as pyrethroids, organophosphates and carbamates. Knowledge of cross-resistance patterns is vital for the development of a successful resistance-management program for whiteflies in the Imperial Valley.

## 2 MATERIALS AND METHODS

### 2.1 Whitefly colonies

A number of *B. argentifolii* strains, two laboratory and seven field, were used for this study.

#### 2.1.1 Susceptible strain (REF)

A silverleaf whitefly colony originally collected in November 1990 on broccoli (*Brassica oleracea italica* Plenck) in the Imperial Valley was maintained in a pesticide-free greenhouse on beans (*Phaseolous vulgaris* L.) at the University of California, Riverside, CA. A subset from this colony was started in 1992 in Brawley, CA<sup>11</sup> on cotton (*Gossypium hirsutum* L. cv. 'Deltapine 5401'). This colony was maintained free of any insecticide exposure. The REF strain was used to meet objective 1 and serve as a baseline to compare the susceptibilities of whiteflies from the selected resistant strain as well as the field populations to selected insecticides and to calculate resistance ratios (RR). The REF strain was tested every alternate generation with imidacloprid to re-confirm the stability of the strain and as a check on the consistent performance of the bioassay procedure.

### 2.1.2 Imidacloprid-selected strain (IM-R strain)

A resistant strain of *B. argentifolii* was developed by selectively breeding for resistance to imidacloprid. Whitefly pupae on melon (*Cucumis melo cantaloupensis* Naud.) leaves were originally collected in May 1993 from imidacloprid-treated cantaloupe fields in Brawley, CA. Imidacloprid was applied as a soil drench at the rate of 0.1 lb acre<sup>-1</sup> (0.11 kg ha<sup>-1</sup>) twice during the season. Approximately 12 000 to 15 000 adult whiteflies were collected on emergence over a two-week period and caged on young cotton plants to initiate an imidacloprid-selected strain.

### 2.1.3 Field strains

Seven field strains from various locations in Imperial Valley, California, were collected from commercial melons, lettuce and cole crops for monitoring purposes. Adults were tested for susceptibility to imidacloprid. In late fall of 1995 and early spring of 1996, populations were sampled and bioassayed to determine the status of resistance in whitefly from broccoli and melon fields that had been treated with imidacloprid. Adults were collected directly from the leaves of the host plant with a battery-operated vacuum sampler<sup>12</sup> and transported to the laboratory. Bioassays were conducted within 2 h after collection.

## 2.2 Techniques

### 2.2.1 Selection method

The first step of this study was to assess development of resistance to imidacloprid under selection pressure in adult *B. argentifolii*. The selection procedure involved growing cotton individually in 4-in plastic pots containing 135 g of a potting mix of peat, perlite and vermiculite (Redi-earth®; Scott's Co.). Concentrations of imidacloprid that gave 40–50% mortality over a number of generations were applied directly to the soil around the main stem. Following a 48-h period to allow for uptake and translocation of imidacloprid, the treated plants were transferred to the colony for exposure to whiteflies. The number of adults subjected to selection in each generation varied depending on the number and vigor of the adult whiteflies of the preceding generation, but was usually about 10 000 to 15 000 in each generation. The treatments did not affect the growth and vigor of the surviving individuals. The eggs that were laid by survivors which developed through to the adult stage were exposed to treated plants. The first three generations of whiteflies, F<sub>1</sub> through F<sub>3</sub>, were selected by exposure of adults to imidacloprid-treated plants at a lethal dose of >LC<sub>30–40</sub> (0.01 mg ml<sup>-1</sup>) (Table 1), so as to obtain survivors to maintain a vigorous colony. The subsequent generations were selected with imidacloprid concentrations that would produce 40–50% mortality among them. This strain has been maintained in the greenhouse under selection every gen-

eration and with increasing concentrations (0.01 to 5 mg ml<sup>-1</sup>) of imidacloprid in every other generation for 32 generations. We monitored responses to selection on a regular basis.

There were four generations of the IM-R strain, F<sub>11</sub>, F<sub>12</sub>, F<sub>29</sub> and F<sub>30</sub>, where selection with imidacloprid was withdrawn. This relaxation or withdrawal of selection pressure was necessary to preserve the colony and it probably retarded the progression of resistance. The colony was infested by mites at these various times and selection was withdrawn to allow regeneration of the colony on new plants.

### 2.2.2 Soil systemic bioassay

Susceptibility to imidacloprid was measured initially in whiteflies with a soil systemic bioassay. Similarly to the resistance selection process, cotton plants were grown individually in 4-in plastic pots with 135 g of the potting mix. When the plants were in the two-true-leaf stage, 10 ml each of five or six concentrations of imidacloprid were applied directly to the soil around the main stem in individual pots. Following a 48-h uptake period, 30 adult whiteflies were exposed to individual leaves in clip cages. A 24-h exposure period was allowed and then mortality was assessed. The criterion for mortality was the inability of an adult to fly when probed with a needle.

The rise of resistance was monitored using the soil systemic assay for generations F<sub>1</sub> through F<sub>15</sub> and for subsequent generations, using the hydroponic bioassay technique.

### 2.2.3 Development of a hydroponic bioassay

A hydroponic bioassay was developed for measuring responses of whiteflies to imidacloprid and is described by Prabhaker *et al.*<sup>13</sup> The bioassay system was modified and consisted of five basic steps: (1) germination of seeds, (2) transfer of seedlings to the aerated hydroponic medium for growth, (3) transfer of plants from the hydroponic medium to a plastic container (capacity 1250 ml) with various concentrations of imidacloprid for uptake over a 24-h period, (4) a 24-h exposure of whiteflies to the systemically treated leaves in clip cages and (5) mortality assessment.

**2.2.3.1 Seed germination.** Cotton (Deltapine) seeds were placed in vermiculite and allowed to germinate at 32°C. Seeds having 1- to 2-in hypocotyls in 48 h were transferred to a hydroponic tank for further growth.

**2.2.3.2 Hydroponic tank.** The hydroponic tank was a plastic container, 8 × 28 × 5 in. Each tank accommodated up to six Plexiglass sheets that spanned the width of the tank, supported by the tank sides. A 2.5-in long aqua-pik was inserted into each of six 0.5-in holes equally spaced on each Plexiglass sheet. Seedlings were carefully placed into each aqua-pik supported by their cotyledons above it. The roots of each seedling extended

**TABLE 1**  
Response of a Colonized Field Strain of *Bemisia argentifolii* to Selection with Imidacloprid

Generation No.	Conc. (mg ml <sup>-1</sup> )	Nos selected	LC <sub>50</sub> (mg ml <sup>-1</sup> ) (95% CI)	Slope (± SE)
REF strain <sup>a</sup>	—	860 <sup>b</sup>	0.042 (0.008–0.051)	2.4 (± 0.14)
Parental	0.01	12 000–15 000	0.023 (0.004–0.081)	1.5 (± 0.21)
F <sub>1</sub>	0.01	10 000–12 000	0.091 (0.003–0.181)	1.4 (± 0.39)
F <sub>2</sub>	0.01	10 000–12 000	0.105 (0.009–0.371)	1.3 (± 0.19)
F <sub>3</sub>	0.01	8000–11 000	0.092 (0.007–0.272)	1.5 (± 0.24)
F <sub>4</sub>	0.03	9000–10 000	0.180 (0.064–0.252)	1.5 (± 0.10)
F <sub>5</sub>	0.03	10 000–11 000	0.369 (0.110–0.581)	1.5 (± 0.12)
F <sub>6</sub>	0.05	9000–10 000	0.258 (0.120–0.590)	1.4 (± 0.11)
F <sub>7</sub>	0.05	12 000–13 000	0.460 (0.183–0.752)	1.5 (± 0.09)
F <sub>8</sub>	0.07	10 000–11 000	0.313 (0.141–0.460)	1.8 (± 0.26)
F <sub>9</sub>	0.07	9000–10 000	0.402 (0.144–0.662)	1.7 (± 0.27)
F <sub>10</sub>	0.10	11 000–12 000	0.670 (0.205–0.902)	1.5 (± 0.21)
F <sub>11</sub>	—	No selection	0.509 (0.158–0.954)	1.8 (± 0.16)
REF strain <sup>a</sup>	—	920 <sup>b</sup>	0.061 (0.031–0.090)	2.5 (± 0.19)
F <sub>12</sub>	—	No selection	0.770 (0.442–1.031)	2.1 (± 0.16)
F <sub>13</sub>	0.07	7000–9000	0.879 (0.342–2.684)	1.8 (± 0.19)
F <sub>14</sub>	0.10	10 000–11 000	0.891 (0.432–3.224)	1.9 (± 0.19)
F <sub>15</sub>	0.10	9000–10 000	1.059 (0.412–2.290)	2.3 (± 0.15)
REF strain <sup>a</sup>	—	658 <sup>b</sup>	0.041 (0.027–0.084)	2.7 (± 0.14)
F <sub>16</sub>	0.30	10 000–11 000	1.392 (0.505–3.220)	2.1 (± 0.25)
F <sub>17</sub>	0.30	9000–10 000	1.327 (0.843–3.381)	2.2 (± 0.16)
REF strain <sup>a</sup>	—	720 <sup>b</sup>	0.060 (0.034–0.086)	2.6 (± 0.19)
F <sub>18</sub>	0.50	8000–9000	2.593 (1.809–3.928)	2.3 (± 0.18)
F <sub>19</sub>	0.50	10 000–12 000	2.734 (1.044–3.320)	2.1 (± 0.15)
F <sub>20</sub>	0.70	11 000–12 000	2.942 (0.942–3.815)	2.2 (± 0.14)
F <sub>21</sub>	0.70	10 000–11 000	3.382 (1.404–4.891)	2.3 (± 0.16)
REF strain <sup>a</sup>	—	780 <sup>b</sup>	0.079 (0.043–0.175)	2.8 (± 0.18)
F <sub>22</sub>	1.0	10 000–11 000	4.706 (3.100–7.642)	2.2 (± 0.17)
F <sub>23</sub>	1.0	14 000–16 000	4.671 (0.180–9.269)	2.5 (± 0.23)
REF strain <sup>a</sup>	—	840 <sup>b</sup>	0.072 (0.064–1.120)	2.8 (± 0.18)
F <sub>24</sub>	2.0	11 000–12 000	5.648 (4.974–8.942)	2.7 (± 0.12)
F <sub>25</sub>	3.0	10 000–12 000	5.374 (4.340–7.935)	2.9 (± 0.19)
REF strain <sup>a</sup>	—	920 <sup>b</sup>	0.089 (0.056–1.024)	2.9 (± 0.09)
F <sub>26</sub>	3.0	8000–9000	7.012 (5.478–8.989)	3.0 (± 0.14)
F <sub>27</sub>	5.0	10 000–12 000	7.305 (5.780–8.764)	3.1 (± 0.14)
F <sub>28</sub>	5.0	9000–10 000	7.214 (5.280–8.106)	3.1 (± 0.19)
F <sub>29</sub>	—	No selection	6.986 (5.091–9.980)	2.8 (± 0.21)
F <sub>30</sub>	—	No selection	6.694 (4.620–8.709)	3.0 (± 0.10)
F <sub>31</sub>	5.0	9000–10 000	6.646 (4.976–8.440)	3.1 (± 0.09)
F <sub>32</sub>	5.0	10 000–12 000	6.924 (4.904–7.677)	3.5 (± 0.16)

<sup>a</sup> LC<sub>50</sub> of the REF strain ranged from 0.04 to 0.09 mg ml<sup>-1</sup> through the duration of this study.

<sup>b</sup> Number of whiteflies bioassayed.

through the aqua-pik into the aerated nutrient solution within the tanks and were allowed to grow undisturbed. Seedlings grew into young plants over a three- to five-week period, after which time they were ready for the hydroponic bioassay.

**2.2.3.3 Bioassay technique.** When cotton plants had reached the two-true-leaf stage, their supporting Plexiglass sheets were lifted off of the hydroponic culture

tank and transferred individually to plastic food containers holding 1000 ml of water and 30 ml of a specific concentration of imidacloprid. Each container held four to six plants simultaneously. Plants were allowed uptake of imidacloprid for a period of 24 h. At least five concentrations of imidacloprid that produced 5–95% whitefly mortality were used for each test. Control plants were exposed to water alone. Following the uptake period, 30–40 adult whiteflies were transferred to clip cages attached to leaves on the treatment plants.

Clip cages had diameters of 8 cm and were fitted with polyurethane foam collars to avoid whitefly escape. Mortality was determined after 24 h following the same criterion described above.

#### 2.2.4 Yellow sticky card technique

To evaluate for cross-resistance in the IM-R strain to bifenthrin, endosulfan, chlorpyrifos and methomyl, a yellow sticky card technique was used. This technique was developed by Prabhaker *et al.*<sup>11,14</sup> for monitoring whitefly resistance to conventional insecticides. Briefly, each yellow card (7.5 × 12.4 cm) was first sprayed with a thin layer of insect adhesive (Tanglefoot, MI) from an aerosol spray can. Next, a series of concentrations of each insecticide was sprayed on the cards using a Potter Spray Tower.<sup>15</sup> At least six concentrations were used to produce a range of mortality from 5 to 95%. Controls were sprayed with water alone. Treated cards were exposed for 30 s to adult whiteflies confined within the various cages or long enough to collect 50–100 adults per concentration. Cards containing the IM-R adults were then placed on a styrofoam slab (20 × 10 cm) in an ice chest containing 3.8 litre of water to maintain humidity at ≥90% at room temperature. Mortality of the adults was determined after 24 h by checking the whiteflies under a microscope for movement by probing them with a needle.

Results obtained from the dose-mortality experiments with all three types of bioassays were analysed using the probit model.<sup>16–17</sup> Failure of the 95% CI to overlap at LC<sub>50</sub> was used as the criterion to indicate significant differences ( $P < 0.05$ ). Resistance ratios were determined by dividing the LC<sub>50</sub> of the IM-R and field strains by the LC<sub>50</sub> determined by the latest bioassay results of the REF strain.

### 2.3 Insecticides

Technical grade imidacloprid was obtained from the manufacturer (Bayer, Kansas City, MO) both for selection of a resistant strain and for susceptibility tests. Technical material was diluted in acetone to a 10 g litre<sup>-1</sup> stock solution and used for necessary dilutions with water.

Commercial formulated insecticides used for treating yellow cards to evaluate cross-resistance were bifenthrin 240 g litre<sup>-1</sup> EC ('Capture 2 EC', a pyrethroid), endosulfan 357 g litre<sup>-1</sup> EC ('Thiodan 3 EC', a cyclodiene), both from FMC, Princeton, NJ; chlorpyrifos 480 g litre<sup>-1</sup> EC ('Lorsban 4 E', an organophosphate; Dow Elanco, Indianapolis, IN) and methomyl 900 g kg<sup>-1</sup> SP ('Lannate', a carbamate, Dupont, Wilmington, DE). Serial dilutions of these materials to the desired concentrations were made in water for application on the yellow cards. Units for imidacloprid are presented as

mg ml<sup>-1</sup> and for the formulated insecticides, units are presented as µg AI ml<sup>-1</sup>.

## 3 RESULTS

### 3.2 Selection for resistance to imidacloprid

At the initiation of selection by imidacloprid, a field strain (parental generation) was bioassayed using the soil systemic assay and found not to differ significantly from the REF strain in response to imidacloprid. Table 1 shows the history of selection of the field strain (IM-R strain) from July 1993 when it was first established, through February 1996. The development of the resistance pattern is presented in Fig. 1.

An increase in resistance was observed in LC<sub>50</sub> values, both for increasing concentrations of imidacloprid and increased selection time (Table 1). The resistance ratio rose to about 4-fold by generation F<sub>4</sub> as a result of these selections (Fig. 1). These results show that the IM-R strain was exposed to only four generations of selection pressure before resistance developed, albeit at a low level of 9-fold in F<sub>5</sub>. But by generation F<sub>16</sub>, the IM-R strain showed a substantial increase in resistance ratio from the F<sub>1</sub> generation of a level of 2-fold to 34-fold in F<sub>16</sub>. This represents an increase in LC<sub>50</sub> from 0.091 to 1.392 mg ml<sup>-1</sup> by the F<sub>16</sub> generation. Resistance to imidacloprid in whiteflies rose and fell over time before reaching a peak of 78-fold by generation F<sub>24</sub>. High-level resistance in this strain appeared by the F<sub>27</sub> generation at 82-fold. After selection pressure for 32 generations, the LC<sub>50</sub> value of imidacloprid was 6.924 mg ml<sup>-1</sup>, indicating a resistance level of 78-fold.

Resistance to imidacloprid developed at a slow rate with some fluctuations during the first few generations, (F<sub>1</sub> to F<sub>15</sub>, maximum RR = 17-fold) followed by a gradual increase at a fairly rapid rate in the succeeding generations (F<sub>16</sub> to F<sub>32</sub>; RR from 34- to 78-fold). The resistance to imidacloprid stabilized at c. 30-fold during generations F<sub>16</sub> and F<sub>17</sub>. The rise of adult resistance to imidacloprid followed a predictable pattern described by Georgiou<sup>18</sup> and Sawicki.<sup>19</sup> Rate of development of resistance is gradual initially in a population in which the R (resistance) genes are rare and subsequently at an accelerated rate to a higher level based on the expression of R genes in the resistant homozygote.

The initial slope value of the parental strain was 1.5 for imidacloprid, indicating a high degree of heterogeneity of response. However, as selection pressure was applied, slope values fluctuated, indicating an increase in heterogeneity of the selected strain. Beginning with generation F<sub>15</sub> and thereafter, slope values stabilized (2.3 to 3.0) indicating a more homogenous condition, increasing in the final selections to 3.5.

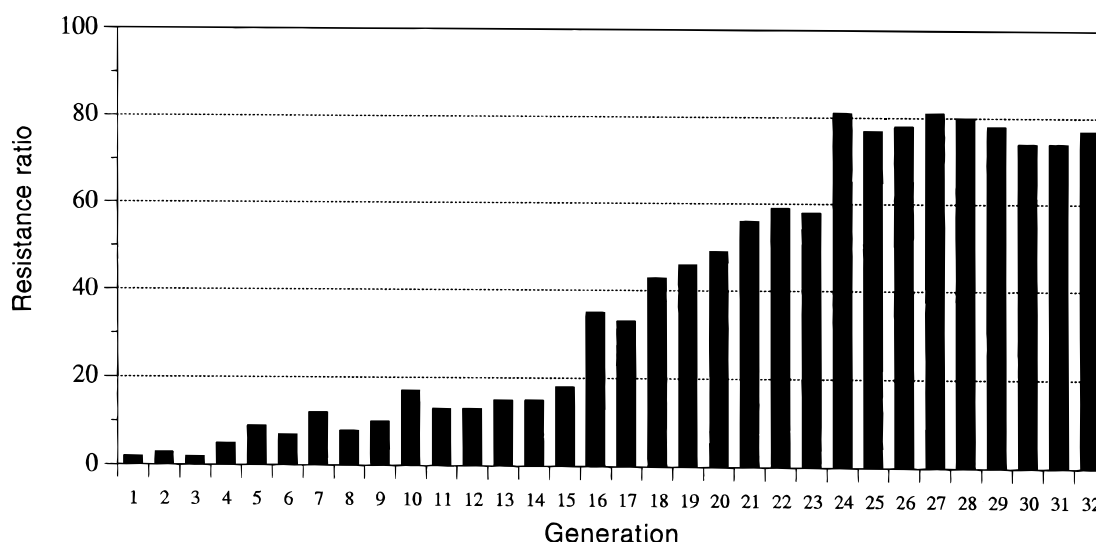


Fig. 1. Progression of resistance to imidacloprid in a laboratory selected colony of *Bemisia argentifolii* for 32 generations.

### 3.2 Evaluation of the hydroponic bioassay

The  $LC_{50}$  values of the IM-R strain during the selection process in generations  $F_{15}$  through  $F_{32}$  were determined using the hydroponic system. A comparison of the two bioassays, soil and hydroponic, was made simultaneously for two generations,  $F_{16}$  and  $F_{17}$ , to examine the differences in response of whiteflies to imidacloprid (Table 2). Results show that the  $LC_{50}$  values are comparable by both methods with no significant differences, although slight differences were observed in the resistance levels for the two generations. The RR values obtained were 3- to 5-fold less with the hydroponic bioassay than with the soil bioassay, indicating that the increase in resistance from generation  $F_{16}$  was real and not an artefact of the change in bioassay. The observed values clearly show the fluctuations in the

levels of susceptibility to imidacloprid over time relating to heterogeneity in the population. The hydroponic system was sufficiently sensitive to detect the changes in susceptibility to imidacloprid.

#### 3.2.1 Baseline susceptibility

The  $LC_{50}$  values varied slightly from field to field. Monitoring of seven different populations of adult whiteflies collected in Imperial Valley, CA, using the hydroponic bioassay, confirmed significant differences in  $LC_{50}$  values. Dosage-mortality and resistance ratios for imidacloprid for these seven field populations are presented in Table 3. The  $LC_{50}$  values of various fields ranged from 0.002 to 0.926 mg ml<sup>-1</sup>, showing low to moderate levels of resistance (0.02 to 15-fold). The highest  $LC_{50}$  value was observed in field #7 at 0.926 mg ml<sup>-1</sup> displaying a resistance ratio of 15-fold. Differences in sus-

TABLE 2  
Comparison of Responses of Whiteflies to Imidacloprid with Soil and Hydroponic Bioassay

Type of bioassay	Strain	Generation	n	$LC_{50}$ (mg ml <sup>-1</sup> ) (95% CI)	Slope ( $\pm$ SE)	RR <sup>a</sup>
Soil	IM-R	16	680	1.952 (0.865–3.761)	2.1 ( $\pm$ 0.12)	37.0
Soil	IM-R	17	590	2.016 (1.082–4.220)	2.2 ( $\pm$ 0.26)	38.0
Soil	REF	—	510	0.053 (0.029–0.396)	2.5 ( $\pm$ 0.15)	—
Hydroponic	IM-R	16	740	1.392 (0.505–3.220)	2.1 ( $\pm$ 0.25)	35.0
Hydroponic	IM-R	17	660	1.327 (0.843–3.381)	2.2 ( $\pm$ 0.16)	33.0
Hydroponic	REF	—	658	0.04 (0.027–0.084)	2.7 ( $\pm$ 0.14)	—

<sup>a</sup> RR (resistance ratio) =  $LC_{50}$  of IM-R strain/ $LC_{50}$  of REF strain.

**TABLE 3**  
Toxicity of Imidacloprid to *Bemisia argentifolii* Adults collected on Broccoli and Melons from Imperial Valley, CA, with the Hydroponic Bioassay

Field	Collection date	n	LC <sub>50</sub> (mg ml <sup>-1</sup> ) (95% CI)	Slope (±SE)	RR <sup>a</sup>
REF	Feb '96	920	0.089 (0.051–1.023)	2.9 (±0.09)	—
IM-R	Feb '96	1250	6.924 (4.904–7.672)	3.5 (±0.15)	78.0
REF	Sept '96	860	0.081 (0.04–0.25)	2.6 (±0.23)	—
1	Sept '96	676	0.002 (0.0008–0.007)	1.6 (±0.45)	0.02
2	Oct '96	564	0.053 (0.015–0.093)	1.8 (±0.22)	0.6
3	Oct '96	880	0.074 (0.031–0.166)	1.9 (±0.43)	0.9
4	Nov '96	750	0.512 (0.134–0.857)	2.0 (±0.24)	6.0
REF	March '96	780	0.062 (0.036–0.093)	2.8 (±0.18)	—
5	March '96	876	0.217 (0.094–0.525)	2.1 (±0.18)	3.5
6	April '96	790	0.408 (0.116–0.824)	1.8 (±0.26)	7.0
7	April '96	656	0.926 (0.872–3.447)	1.9 (±0.32)	15.0

<sup>a</sup> RR (Resistance ratio)=LC<sub>50</sub> of IM-R & field strains/LC<sub>50</sub> of REF strain.

**TABLE 4**  
Cross-Resistance of the IM-R Strain of *Bemisia argentifolii* to Selected Insecticides

Insecticide	Strain	n	LC <sub>50</sub> <sup>a</sup> (95% CI)	Slope (±SE)	RR <sup>b</sup>
Imidacloprid	IM-R	560	6.646 (4.976–8.440)	3.1 (±0.09)	75.0
	REF	920	0.089 (0.056–1.024)	2.9 (±0.09)	—
Bifenthrin	IM-R	550	2.525 <sup>b</sup> (1.923–4.272)	1.8 (±0.18)	7.0
	REF	680	0.370 (0.261–0.492)	3.2 (±0.35)	—
Chlorpyrifos	IM-R	640	1.253 <sup>b</sup> (0.870–1.883)	3.1 (±0.45)	1.0
	REF	720	0.954 (0.785–2.252)	3.0 (±0.29)	—
Endosulfan	IM-R	620	0.826 <sup>b</sup> (0.504–1.172)	2.8 (±0.19)	1.5
	REF	660	0.562 (0.436–0.753)	3.1 (±0.34)	—
Methomyl	IM-R	580	0.081 <sup>b</sup> (0.042–0.138)	4.2 (±0.33)	0.4
	REF	870	0.182 (0.095–0.338)	2.9 (±0.53)	—

<sup>a</sup> LC<sub>50</sub> units for imidacloprid are expressed in mg ml<sup>-1</sup>, LC<sub>50</sub> units for bifenthrin, chlorpyrifos, endosulfan and methomyl are in µg AI ml<sup>-1</sup>.

<sup>b</sup> RR (Resistance ratio)=LC<sub>50</sub> of IM-R strain/LC<sub>50</sub> of REF strain.

ceptibility of whiteflies under imidacloprid treatments were apparent using the hydroponic bioassay. The  $LC_{50}$  for the whiteflies from field #1 was significantly lower (0.002) than that of the REF strain (0.062–0.089 mg ml<sup>-1</sup>), showing that imidacloprid treatments were probably effective in that field. The results indicated that there is no widespread resistance to imidacloprid in whitefly populations from Imperial Valley. Our study shows that the field populations in Imperial Valley still have fairly low  $LC_{50}$  values compared to the selected IM-R strain with the highest  $LC_{50}$  value of 6.924 mg ml<sup>-1</sup>. Although our data indicated a decrease in susceptibility to imidacloprid in field #7, a reduction in field control was not reported in any of the fields in Imperial Valley. In general, adequate control of whiteflies in these fields is being achieved using imidacloprid alone.

### 3.3 Cross-resistance evaluation

Estimated resistance levels of the IM-R strain as compared with the susceptible strain using the yellow sticky card technique are shown in Table 4. Results showed the IM-R strain was not resistant to endosulfan and chlorpyrifos. Resistance levels ranged from 1- to 1.5-fold (Table 4) for the two chemicals. This agrees with the tendency of cross-resistance to be confined to compounds within a single class in general, i.e. not expanding to OPs, carbamates and pyrethroids. No cross-resistance was observed to methomyl (RR = 0.4) indicating that there is no correlation between resistance to imidacloprid and selected carbamate compounds in *B. argentifolii*. However, tests with the pyrethroid, bifenthrin, showed that bifenthrin was about 7-fold less toxic to the IM-R whiteflies than the susceptible (REF) whiteflies. These results suggest that the mechanism of resistance to imidacloprid in whiteflies, which is not known at the present time, may overlap with one or more mechanisms that confer bifenthrin resistance.

## 4 DISCUSSION

The delay in rapid increases in resistance levels to imidacloprid may be due to the low frequency of the resistance gene<sup>20</sup> in this strain. However, after 15 generations of selection the strain had developed >30-fold resistance to imidacloprid. The slow and steady rise of resistance suggests the involvement of a polygenic system. Further selection with imidacloprid led to higher levels of resistance, with the greatest resistance ratio recorded at 82-fold in generation 27. However, when selection was withdrawn at generations F<sub>29</sub> and F<sub>30</sub> due to mite problems, a small (non-significant) drop in resistance level (to 75-fold) for the two generations was observed.

It is noteworthy that the initial attempt to select a field strain for resistance to imidacloprid was successful. This may indicate a natural tolerance to imidacloprid, perhaps due to exposure of these field populations to the various insecticides used previously in Imperial Valley. Moreover, there may have been a pre-selection for tolerance in the imidacloprid-treated melon field that yielded parents for the greenhouse selection procedure. The initially large genetic pool represented in the field collection probably enhanced the selection for resistance. An insecticide-resistant population can be derived only where individuals with pre-existing genes for resistance increase under selection pressure.<sup>21</sup> A comparison with the  $LC_{50}$  values of the REF strain failed to reveal any resistance to imidacloprid in the original parental strain (RR = 0.5), although resistance genes must have been present in low frequencies based on the subsequent rise in resistance.

At the end of the selection process, slopes of probit regressions were much steeper, compared to the low slope of 1.5 in the parental generation, followed by fluctuations, suggesting that the effect of imidacloprid selection reduced resistance variance in the IM-R strain. This slope pattern is characteristic of true resistance development as reported by Hoskins and Gordon.<sup>22</sup> Regression lines become shallower under application of selection pressure, after which the lines become steeper as resistance genotypes increase in the new population. Based on the slope of 3.5 (relatively low) at the end of the selection process, it is possible that the IM-R strain can become more homogenous under further selection in the greenhouse with no immigration of whiteflies from outside sources.

Laboratory selection experiments may not provide accurate prediction on the evolution of resistance under field conditions.<sup>23</sup> Nevertheless, our study shows clearly the potential for development of resistance to imidacloprid in natural populations of whiteflies under field conditions, especially because of their rapid development rate and high reproductive potential. These results may be more significant with regard to the potential for imidacloprid resistance to develop in whiteflies under commercial greenhouse conditions. Immigration of whiteflies into high-production greenhouses is limited, thus reducing the potential reservoir of susceptible genes. Alternative host plants are a valuable source of susceptible individuals.<sup>24</sup> These results suggest that due to the systemic action of imidacloprid products, resistance could progress rapidly in whiteflies in ornamental host plants if selection pressure was maintained.

The range of  $LC_{50}$  values observed among the seven field populations probably reflected natural variability in whiteflies. The moderately resistant population (# 7) indicated the presence of a low frequency of resistant individuals that may affect further responses in the populations under constant exposure to imidacloprid. This is not surprising since imidacloprid has been in use



for two years in Imperial Valley, and, given the systemic nature of this compound, resistance is likely to occur with frequent use. The greenhouse selection results indicate a high propensity for resistance in this species and therefore routine monitoring should be maintained to detect changes in susceptibility to imidacloprid.

The hydroponic bioassay technique was sensitive in detecting variable responses among different populations of whiteflies and may be useful as a monitoring technique. Long-term control of whiteflies with imidacloprid might remain effective longer if susceptibility changes were monitored routinely. The hydroponic bioassay could be further extended for use with other systemics for whiteflies, as well as for other homopteran insects and also chewing insects.<sup>14</sup>

The systemic soil bioassay used for monitoring the changes in the rate of resistance development was reliable. However, it was limited for monitoring over a wide area because it was time-consuming (96 h) and required individual plants per concentration. In contrast, results can be obtained in 48 h using the hydroponic bioassay using multiple plants to sample a number of fields per dose simultaneously. This technique will permit more extensive and rapid surveys in future for monitoring in Imperial Valley. However, both methods of bioassay can be used for resistance monitoring since whiteflies responded similarly (based on non-overlap of CIs of LC<sub>50</sub> values.)

Selection with chemicals can result in cross-resistance to related compounds. Although imidacloprid is not related structurally to carbamates, organophosphates and pyrethroids, once imidacloprid resistance had become established in the IM-R strain, it was important to determine whether resistance would extend towards these other classes of insecticides used for controlling whiteflies. If resistance is due to detoxification of imidacloprid by enzymatic attack, this might be of concern because this is known to occur with organophosphates and pyrethroids in whiteflies.<sup>25–27</sup> Our results indicate that selection with imidacloprid resulted in limited (7-fold) resistance to bifenthrin but did not confer cross-resistance to endosulfan, chlorpyrifos or methomyl. Because of the reduced potential for cross-resistance to these three compounds, it is conceivable to include them in a rotation scheme to manage whitefly resistance to imidacloprid.

In summary, the results of this study indicate that the hydroponic technique is a feasible bioassay for obtaining baseline data of whitefly populations. Presently, resistance to imidacloprid does not appear to be a problem, but routine monitoring should be maintained to document the changes in effectiveness of imidacloprid to manage resistance by implementing alternative control measures. It is evident from our study that the risk for imidacloprid resistance in this economically important species must be given serious consideration.

## 5 ACKNOWLEDGEMENTS

The authors wish to thank M. J. Blua and J. Immaraju for critical comments on the manuscript. Thanks also to Bayer Ag. Company (Kansas City, MO) for financial support for part of this work.

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